Time-dependent Modulation of Cerebral Ischemic Injury by Activated Macrophages/microglia after Lipopolysaccharide Microinjection into Rat Corpus Callosum

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tIn brain ischemic insult, inflammatory cells such as macrophages and lymphocytes are chemo-attracted into the brain lesion and release cytokines, resulting in an activation of microglia that are functionally equivalent to peripheral macrophages in the central nervous system. In cerebral ischemic insults, activated inflammatory cells such as microglia and macrophages may be implicated in the pattern and degree of ischemic injury by producing various bioactive mediators. Here we provide the evidence that activated microglia/ macrophages exacerbate cerebral ischemic injury by over-expression of inducible nitric oxide synthase (iNOS) and pro-inflammatory cytokines. To activate microglia/macrophages, a potent inflammation inducer lipopolysaccharide (LPS, 5 μg/5 μl) was microinjected into rat corpus callosum. LPS induced many of the features characterizing pro-inflammatory macrophageal and microglial activation. First, marked morphological changes were observed after LPS injection: most macrophages/microglia became strongly ED-1- or Isolectin B4-positive and round-shaped 1 day but ramified 7 days after LPS injection. In LPS-untreated control rats, ischemic injury was little evoked by 2-h middle cerebral artery occlusion (MCAO) followed by 3-h reperfusion. However, pre-injection with LPS 1 day before MCAO markedly increased the ischemic injury (Fig. 1). RT-PCR showed that LPS injection induced iNOS mRNA expression mostly in microglia/macrophages, peaking in intensity at 15 h after LPS injection. The increased ischemic injury in LPS-treated rats was well correlated with iNOS level expressed over 3 orders of magnitude than in LPS-untreated rats (Fig. 2). Seven days after LPS injection, however, the iNOS activity was completely returned to the control level. Immunohistochemical studies showed that iNOS- and nitrotyrosine (a peroxynitrite marker)-positive cells were prominent throughout the infarct area. NOS inhibitors aminoguanidine and N^G-nitroarginine, simultaneously injected with LPS, reduced the iNOS immunoreactivity and infarct volume, especially in penumbra regions (Fig. 3). Total glutathione levels were significantly lower in LPS pre-injected rats than in control ones. RNA protection assay and

RT-PCR showed that LPS injected acutely into corpus calosum rapidly induced mRNA expression for pro-inflammatory cytokines IL-1β and TNF-α, peaking in intensity at 9~15 h after LPS injection. In general, pre-injection with LPS 1 day before MCAO markedly increased the mRNA expression of pro- and anti-inflammatory cytokines (Fig. 4) soon after ischemic insult. However, this increased cytokine expression by ischemia/hypoxia was not observed 7 days after LPS injection. Further defining the role of NO in cerebral ischemic insults would provide the rationale for new therapeutic strategies based on modulation of microglial and macrophageal NO production in the brain.